Agilent Hi-Plex Columns for Superior Resolution of Organic Acids in Wine

Application Note

Food and Beverage

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Introduction

The Agilent Hi-Plex H is a high-performance ligand-exchange chromatography column based on polystyrene/divinylbenzene (PS/DVB) with an 8% crosslinking and hydrogen counter ion. Typically used for the analysis of sugars, sugar alcohols, and organic acids, its monodisperse sulfonated packing gives improved column efficiency, lower column pressure, and assured batch-to-batch reproducibility. The superior separation ability of Hi-Plex H is demonstrated by comparison with a column from another manufacturer, in the analysis and quantification of the organic acids in wine. The quantitative assessment of organic acids is important for the quality control of wine, because the classes and content of organic acids give a characteristic taste to the end product.

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Materials and Reagents

Sample Preparation

Acetic acid, lactic acid, succinic acid, malic acid, citric acid, and tartaric acid are the main organic acids in wine. A standard solution was made up containing these organic acids, along with glucose, fructose, and ethanol. The ten compounds were weighed into the same vial, as shown in Table 1, and dissolved in 10 mL of 0.009 M H_2SO_4. Injection volume was 20 µL.

Table 1. Weight of Ten Compounds

Standard constituent	Amount (g)
1. Citric acid	0.0141
2. Tartaric acid	0.0283
3. Glucose	0.0120
4. Malic acid	0.0352
5. Fructose	0.0543
6. Succinic acid	0.0201
7. Lactic acid	0.0193
8. Glycerol	0.0969
9. Acetic acid	0.0450
10. Ethanol	0.2127

Both the Shiraz wines analyzed in this study, one rosé and one red, were directly injected without sample pretreatment to allow full identification of all the major components in each one. Twenty µL of each were injected at a time.

Conditions

Columns	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830) Alternative crosslinked PS/DVB, 7.8 × 300 mm, 9 µm
Mobile phase	0.004 M H_2SO_4
Flow rate	0.4 mL/min
Temperature	75 °C
Detector	RI

Results and Discussion

The results of this investigation highlight differences in the selectivities of the Agilent Hi-Plex H column and the column from another manufacturer. As can be seen in both the reference standard (Figure 1) and the wine samples (Figures 2 and 3), the alternative column is less retentive than Hi-Plex H and is unable to separate malic acid and glucose to any great extent, whereas glucose, malic acid, and fructose are equally spaced (with maximum possible resolution) by a Hi-Plex H column.

Exact quantitation of the organic acids and sugars in these wine samples can be achieved by preparing four aqueous calibration standards (containing the ten compounds listed in Table 1), covering a broad concentration range. After injecting each of these into the RI, the resulting peak heights can be determined and plotted against concentration. The slopes of these lines are the response factors for each compound, and the linear correlation coefficients and y-intercepts provide a measure of analytical precision. From this information, the exact concentration of each compound in the wine samples can be determined. Based on the selectivity of these two columns, it is much easier to quantify the exact concentration of each compound when the sample is run on Hi-Plex H, as the co-elution problems of the alternative column make it very difficult to determine the peak heights of malic acid and glucose.

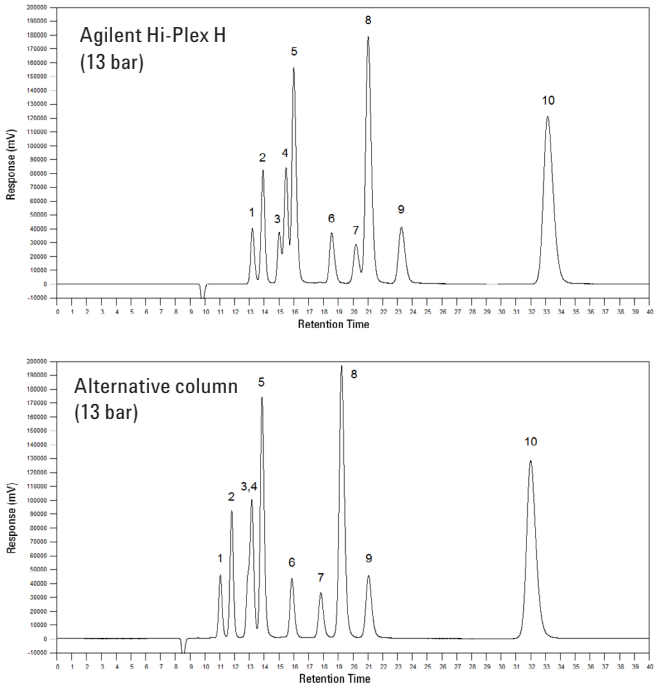

Figure 1. Plots of standard solution on an Agilent Hi-Plex H column (above) and on an alternative column (below). Note the superior discrimination of the Hi-Plex H column, specifically with peaks 3 (glucose) and 4 (malic acid).

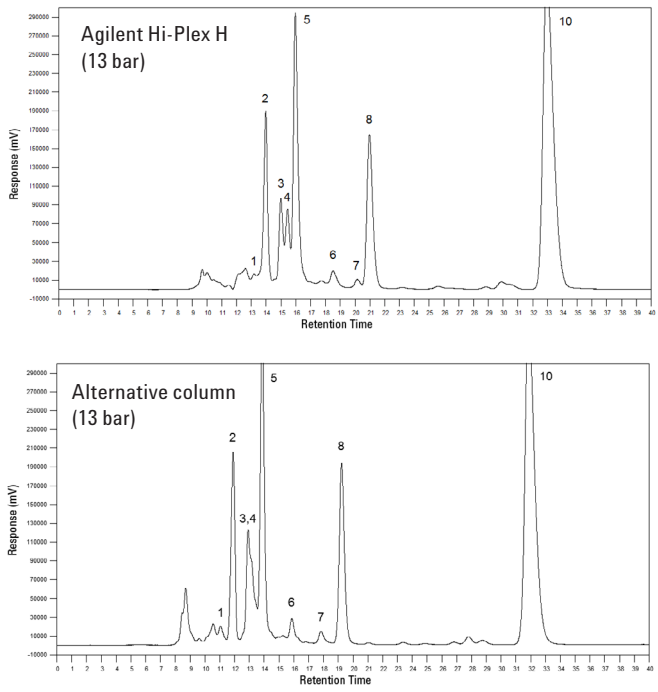

Figure 2. A sample of Shiraz rosé wine on an Agilent Hi-Plex H column (above) and on an alternative column (below).

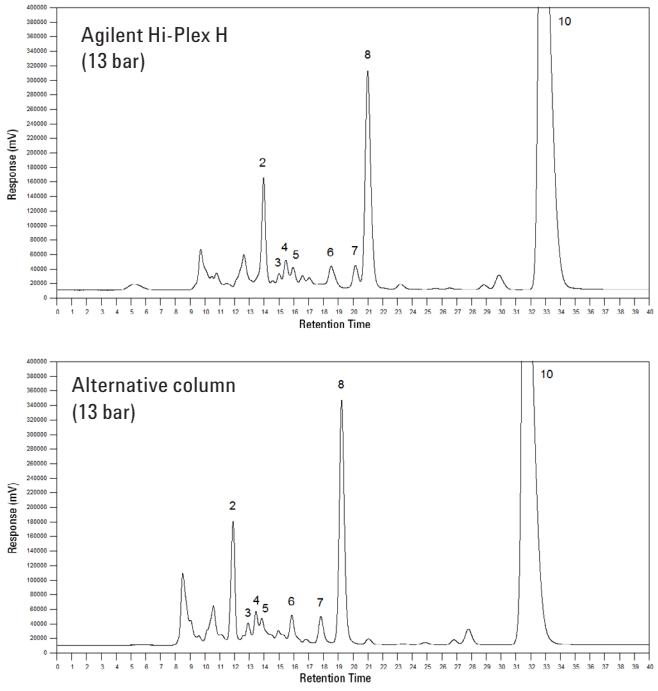

Figure 3. A sample of Shiraz red wine on an Agilent Hi-Plex H column (above) and on an alternative column (below).

Conclusion

The analysis of wines demonstrates how Agilent Hi-Plex H columns provide optimum resolution of closely eluting compounds, enabling quantitation of each. These columns are ideal for the analysis of sugar alcohols and sugar molecules, using water as the mobile phase. Hi-Plex H is also the column of choice for the analysis of organic acids, using dilute mineral acid as eluent. By using the columns at higher operating temperatures, closely eluting compounds can be resolved.

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© Agilent Technologies, Inc., 2011
Published in USA, June 30, 2011
SI-01152

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